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THE FORMS OF PHOSPHORUS IN FORAGE CROPS AS INFLUENCED BY PHOSPHORUS FERTILIZATION

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THE EFFECT OF FERTILIZERS UPON THE FORMS OF PHOSPHORUS AND THE AMOUNTS OF PHOSPHORUS, NITROGEN, AND SILICA IN HAYS.*

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INTRODUCTION.

The compounds of phosphorus in forage plants and grains have been studied extensively during the past 25 to 30 years. In the main this study was confined to the isolation and qualitative study of the phosphorus in organic combination, with some quantitative study of the inorganic and organic fractions in plants. It is with this latter phase of study and methods for determining the same, combined with the variations in these two fractions under various fertilizer treatments, that the present work is concerned.

It is well known that phosphate fertilization of a phosphorus deficient soil causes a marked increase in the phosphorus content of the crops grown upon that soil. However, relatively little exact information is available as to whether or not this increase in phosphorus content is in the organic form or as inorganic phosphates or how the ratios between these forms will change. Accordingly, because of the increasing tendency to use phosphate fertilizers, it seems desirable to make a study as to how much fertilization affects the forms of phosphorus in the plant.

The work was made possible by the assistance of the Soils Department of Minnesota in furnishing a part of the samples used. Work has been carried on for some time by this Department on a low phosphorus peat soil, using as test plants a mixed crop of alsike clover and timothy. The peat soils plots had sufficient lime and potash, and the plots were treated with increasing applications of phosphorus. This work had shown an increase of total phosphorus content of the hays which was related to the magnitude of the phosphate fertilizer application.

HISTORICAL.

It will be necessary to review in some detail the more important publications, on the organic and inorganic fractions of phosphorus in plants, with special reference to the methods of separating the two fractions.

Plimmer (*33*) lists five groups of organic phosphorus compounds in plants:

1. Phytin, an inositol phosphoric ester, probably a calcium magnesium salt of the ester.

2. Phospholipines, lecithin being an example where the phosphorus occurs as a glycerol ester.

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3. Hexose phosphate, in which a hexose molecule is combined with phosphoric acid.

4. Nucleic acid, in which again a carbohydrate is combined with phosphoric acid and attached to the carbohydrate as a purine base.

5. Phosphoprotein, where the phosphorus is present in some form as yet unknown.

Phytin. In 1895 Palladin reported this compound discovered by him in his work on *sinapis niger,* describing it as a substance soluble in cold water, but insoluble in hot water, rich in phosphorus, containing calcium and magnesium but no nitrogen. He did very little with the compound except mention it, and it remained for Posternak (*34, 35, 36*) to conduct the first real investigation on the compound, and to give it the name phytin. He prepared phytin from a number of sources such as wheat, rape, corn and potatoes, stating that in grains where there is a small percentage of mineral phosphate, phytin forms 70-90 per cent of the total phosphorus, and that phytin exists as a storage reserve material for the supply of available phosphorus to the young embryo.

Subsequent to the study by Posternak (*36*) a good deal of interest was shown by many European workers in the organic phosphorus compounds of plants, without any real contribution to the identification of phytin, and in a resumé on organic phosphorus compounds Rose (*43*) reviews the literature and discusses some of the beliefs that were advanced regarding these compounds. He concludes that many of the statements of the earlier investigators were more or less conjectural, but that undoubtedly inosite phosphoric acid esters play an important rôle in the higher plants.

Forbes (*19*), in connection with the study of the rôle of the various phosphorus compounds in animal nutrition, reviews fairly completely the work previous to 1914 on the various organic compounds of phosphorus and also the methods used in separating the organic and inorganic fractions. The conclusions of early investigators were not always in accord as to the percentage of phytin in the different plants, due mainly to inaccurate methods of determining it. Neither were they agreed as to the use of phytin in the metabolism of the plant.

A good deal of controversy existed as to whether Posternak (*36*) had given a true description of phytin, and Anderson (*5*) gives a review of his attempt to determine the structure of phytin, and to synthesize it. He tried to isolate the compound as the salt of some of the heavy metals from such substances as wheat bran, cottonseed meal, oats and corn. Throughout most of his earlier work he obtained two compounds, the mono-and tri-phosphoric acid esters of inositol*. He discovered that the enzyme phytase in much of his early work was instrumental in giving him split products of phytin, and he concluded that in extracting phytin from the grains, it was necessary to use a 1.0 per cent acid solution to prevent the enzyme action, instead of the 0.2 per cent hydrochloric acid solution used by former workers. Extraction with the weaker solution gave higher results for

*The word inositol has been generally accepted as the correct name of this compound, though inosite is used frequently in connection with the earlier work on these compounds.

inorganic phosphorus due to the splitting of phosphorus from organic combination by the enzyme phytase.

Rather (*39*), while working with the phosphorus compounds of cotton-seed meal in which he considered that he had demonstrated the phosphorus to be entirely in organic combination, found that contrary to Anderson's statement, 0.2 per cent acid solution did prevent the action of phytase to a certain extent. The difference in the results of these investigators may have been due to buffers present in the extracting solution.

Boutwell (*9*), in extracting phytin from Indian mustard and wheat bran, used the method of Clark (*10*) in which 2.0 per cent hydrochloric acid was used for extraction, believing that the 1.0 per cent acid used by Anderson (*3*) did not exclude all enzyme activity.

Many formulae for the structure of phytin were advanced, and much disagreement over methods of preparation, but Kostytschew's (*28*) "Plant Physiology" gives the formula of phytin as a hexa phosphate of inosite, and gives Posternak (*37, 38*) the credit for establishing the true structure of the compound, and he says although phytin is spoken of as a phytochemical compound, a phytin synthesis in light has not been recorded.

Organic compounds other than phytin. Hexose phosphate for the present work may be disregarded as it is so easily hydrolyzed by acid that it would appear as inorganic phosphorus in the extract, if phosphorus were present in such form in forage. The remaining forms of phosphorus will be included in the phospholipines, nucleic acid, and nucleoproteins, and Forbes (*20*) has compiled most of the information before 1914 on these forms of phosphorus in hays.

The data on the nucleic acids and nucleoproteins are listed under different headings due to the fact that it was impossible to ascertain in just what form these exist in the plant. Some authors speak of protein phosphorus, others of nuclein phosphorus, but in the main it may be taken to mean the inert organic phosphorus remaining after deducting the phytin and phospholipine phosphorus fractions.

Fraps and Rather (*21*) investigated the ether extract of several kinds of hay, straw and other roughages. The phosphorus content of the ether extract was taken to represent the phosphatid content of the products. Their results show the lecithin phosphorus content to be extremely low in these fodders.

From a review of the literature on phosphorus compounds it would appear that the phytin fractions and the nuclein or nucleoprotein fractions are the major portions of phosphorus in the organic form that must be considered in this work on hays. There is a possibility of some phosphatid phosphorus being present but it will, in all probability, be small in amount.

Forbes (*19*) in his work on a method for separating the various forms considers only the three fractions, protein phosphorus, phytin, and the inorganic portion. Of these three phytin seems to be found in larger quantities in the seeds of most plants but does not occur to any appreciable extent, in comparison with the nucleoproteins and inorganic phosphorus, in the forage crops.

The differentiation of the organic and inorganic phosphorus. In the earlier work on plant phosphorus the main interest was the organic portions, particularly phytin phosphorus, and less interest was shown in the determination of the inorganic fraction. However, though many of the investigators report inorganic phosphorus, very few of them agree on methods for its determination.

Plimmer (*33*) among the first investigators, used a solution of magnesium citrate to precipitate the inorganic phosphorus as the magnesium ammonium phosphate. This method, however, was later shown to be incorrect in the presence of phytin which is also precipitated by the magnesium citrate solutions. Direct precipitation by ammonium molybdate solution at 65°C. in the presence of dilute nitric acid was used by Hart and Andrews (*23*). The dilute acid was used as a precaution against the hydrolysis of some of the organic forms of phosphorus by the acid. McCollum and Hart (*29*) and Patten and Hart (*31*) also used this method, but here again it was shown that phytin interfered, inhibiting the precipitation of the yellow compound and the organic matter also present in these extracts helped increase the error.

A microchemical method was employed by Hartwell and Hammett (*25*) on turnips in which they added magnesium sulphate and ammonium chloride to a section of the turnip and studied the crystals formed, estimating the phosphorus in this manner. They checked their method against the precipitation by both magnesium mixture and ammonium molybdate and finally concluded that the two latter methods included in the precipitation a part of the organic fraction.

A method in which the inorganic phosphorus was first precipitated by magnesium mixture, then re-dissolved in nitric acid and the phosphorus precipitated with ammonium molybdate and finally determined as the magnesium pyro-phosphate, was used by Vorbrodt (*49*); the inorganic and organic fractions of phosphorus reported by Suzuki and Yoshimura (*46*), Suzuki, Yoshimura and Takaishi (*47*), and Heubner and Rieb (*26*) were determined by some modification of the above methods. The use of acid alcohol, later employed by Forbes, was introduced by Stutzer (*45*).

Forbes and associates (*18*) studied in detail methods for the separation of inorganic phosphorus. They decided that the method used by Hart and Andrews (*23*) was the most specific starting point for further work, and they criticized the method on the following accounts:

1. The time for extraction used by Hart, which was fifteen minutes, was not long enough to extract all the inorganic phosphorus.

2. Ammonium molybdate in neutral solution was found to give poor precipitation, leaving part of the inorganic phosphorus in solution.

3. The bulky flocculent precipitate forming, gave trouble and it was often difficult to know whether or not precipitation was complete.

4. The precipitate was found very difficult to filter.

5. The method involves the precipitation of inorganic phosphorus in the presence of phytin. A quantitative precipitation of inorganic phosphorus alone from a solution containing phytin appears to be impossible.

6. Nucleinic acid may be hydrolyzed by the nitric acid in this precipitation, the result being the formation of inorganic from organic phosphorus.

7. Nucleinic acid, like phytin, appears to hinder the formation of the yellow precipitate.

8. Another possible error is the carrying down of phosphorus containing proteids with the yellow precipitate.

In the work previous to this paper of Forbes, the extracting solution generally used was 0.2 per cent hydrochloric acid. Forbes found this satisfactory but lengthened the time of extraction to three hours. Magnesia mixture was used to precipitate the inorganic phosphorus and the phytin from the extract solution, the nucleinic acids remaining in solution as they are soluble in ammonium hydrate. This precipitation also removes part of the bulky precipitate. The inorganic phosphate was then dissolved from the magnesia mixture precipitate by the use of acid alcohol. This acid alcohol was believed to leave the phytin in the precipitate and remove the inorganic phosphate. Phenol was employed to prevent enzyme action on the phytin.

Experiments were carried out to see if phytin or nucleinic acid would decompose in 0.2 per cent hydrochloric acid. They were unable to demonstrate any decomposition in this reagent in either 3 or 24 hours extraction.

Plimmer (*33*) carried out some work previously on the decomposition of these compounds in different strengths of acids. He states that glycerophosphoric acid is slowly hydrolyzed by dilute acid, complete separation of the phosphoric acid requiring ten days at 92°C. Phytic acid was found to be the most stable of the organic phosphorus compounds. At 37°C. dilute acid causes no hydrolysis; at 64°C. a slight hydrolysis could be detected in 24 hours. Hexose phosphate was found to be easily hydrolyzed. Nucleic acid was found to be hydrolyzed by acid, normal hydrochloric producing complete hydrolysis in eight days at 75°C.

These results indicate that there is little danger of the organic compounds of phosphorus undergoing any hydrolysis in an extracting solution as concentrated as 1.0 per cent hydrochloric acid.

The final method adopted by Forbes and associates was:

1. Three hours extraction with 0.2 per cent hydrochloric acid.

2. Precipitation of the inorganic phosphorus and phytin by adding magnesia mixture and NH_4OH, and allowing to stand over night.

3. Filtering the above precipitate and washing it with 2.5 per cent NH_4OH and then with 95 per cent alcohol until free of ammonia.

4. The inorganic phosphorus was dissolved from the precipitate with 95 per cent alcoholic solution containing 0.2 per cent hydrochloric acid, filtering and precipitating the inorganic phosphorus with ammonium molybdate, finally determining it as the pyrophosphate.

The above method was employed by Ames and Boltz (*2*) in some work on the phosphorus fractions in alfalfa hay, and also by Rather (*41, 42*) who used 5 per cent phenol solution to prevent enzyme action.

Following the work of Forbes and associates on this method, Collison (*11*) suggested a method in which he proposed using an acid alcohol solution for the extraction of the inorganic phosphorus. The method would

prevent enzyme action but he would still have the difficulty of separating the phytin, phosphatid and inorganic phosphorus compounds. He states that the method is not proposed as a perfect means for this analysis but he proposes it as an improvement on some of the others. He is the only person in the literature using this method so it can not have proven a success.

Anderson (*4*), after publishing a large amount of material on the mono- and tri-phosphoric acid esters of phytin, decided to carry out some work on the effect of phytase, and in this connection he ran some inorganic analyses. He found the enzyme very active in 0.2 per cent hydrochloric and he increased his extraction solution to from 0.5 to 1.0 per cent hydrochloric acid, at which concentration he found the enzyme was destroyed. He did not follow the Forbes method of precipitating the inorganic phosphorus with magnesia mixture but used the direct precipitation with ammonium molybdate and reported his results by this method.

Bodnar (*8*), in some similar work, used much the same method as Forbes, i.e. the precipitation by magnesia mixture, but he used 25 cc. of 8 per cent nitric acid in the cold to dissolve the inorganic phosphorus from the magnesia mixture precipitate. The remainder of the determination was carried out by the regular phosphorus method.

Some colorimetric work was carried out on the determination of phytin. Heubner and Stadler (*27*) used a method in which they determined phytin by treating the extract solution with a ferric chloride solution in the presence of ammonium thiocyanate. According to this method the phytin is precipitated from solution as the iron salt of phytic acid, the salt being very slightly soluble in the hydrochloric acid solution. When the phytin is removed from solution the excess iron gives an indication by reacting with the ammonium sulphocyanate.

Averill and King (*7*) used the above method in some determinations on oats, rye, wheat and soybeans. They used 2.0 per cent acid for extraction as they believed the enzyme phytase was still active in the 1.0 per cent acid solution and Rather (*40*) employed the same method on Kafir corn and cottonseed meal. Rather states that 97 per cent of the phosphorus of cottonseed meal is in organic combination in the form of phytin as determined by the Heubner and Stadler colorimetric method. The remaining three per cent was believed to be inorganic, being determined by the Forbes method, but Rather questions the accuracy of this method, and thought it best not to publish results based on it. In an attempt to employ the colorimetric method on the hays of soybean, cow pea, velvet bean and alfalfa, the colour of the extracted solution of these hays prevented the use of such a method.

A review of the literature shows a decided lack of some dependable method for the determination of inorganic phosphorus in vegetable substances.

The Assocation of Official Agricultural Chemists (*20*) took up the matter and they made Forbes chairman of a committee to consider methods for the determinations of inorganic phosphorus in plant and animal tissues. In the report Forbes outlines a method for the determination of inorganic phosphorus of animal tissues that he considered would do as an official method. It was a modification of his former method, but though he outlines

the attempt made on vegetable or plant inorganic phosphorus, he finally concludes that he is unable to recommend it or any other method as a standard method for this work and he gives as one of the main difficulties the separation of the inorganic fraction from the gummy magnesia mixture precipitate.

The Method.

Search for a method. In a review of methods it was hoped to secure one that would give at least comparative results, and that would prove short enough to allow the carrying out of the analysis, in a reasonable time, of the large number of samples collected for this work.

The great amount of work done on the phosphorus of blood, a problem which in a sense is parallel to the vegetable work, suggested the idea that some of the methods used in that work might be adopted, methods that would be more rapid and probably more reliable than the older ones.

The Fiske and Subbarow method (*17*) was therefore tried, using the Dubosque colorimeter, the only type of colorimeter available at that time. It was found, however, in all cases of a hay extract, that the yellowish red colour of the extract so changed the blue phosphorus colour produced as to make the reading of the colorimeter impossible. The yellow hay colour added to the blue phosphorus colour, gave the solution a decidedly green tinge. This was especially true in solutions low in phosphorus where the amount of extract had to be large in order to give colour enough for comparison.

Failing to find the above method applicable, the older methods were resorted to and a rechecking of these was undertaken to give a method best suited to this particular type of work.

Forbes' method (*18*) was first tried, using 1.0 per cent hydrochloric acid, instead of the 0.2 per cent used by him, the higher concentration being used to make certain no enzyme action occurred. The method as outlined proved unsatisfactory. The time for the magnesium mixture precipitation was increased from twelve hours to two days before precipitation seemed complete. The filtration proved slow and difficult and had to be carried out carefully under suction, care being taken at the beginning to allow the pores of the filter to fill before applying the suction; otherwise some of the precipitate was carried through the filter.

Results from the method as outlined by Forbes were unsatisfactory and it was believed at the time to be due to the difficulty as suggested by Forbes of dissolving out the inorganic phosphorus from the gummy precipitate.

Collison's (*11*) method of acid alcohol extraction was then tried, but it did not prove as easily handled as the Forbes method and was finally discarded.

Anderson (*3*), in his work on phytase, used a direct precipitation with ammonium molybdate, and believing from the work of Hart and Tottingham (*24*) that there probably was not much phytin in these extracts to interfere with the yellow precipitate, this method was tried. Poor results were obtained, however, as a muddy brown precipitate was obtained which proved very difficult to filter. The attempt was made to redissolve this first precipitate

in ammonia and precipitate again, but the second precipitation did not seem to reduce the errors in any way.

In connection with this method an attempt was made to determine the phytin phosphorus in the extract, using the Heubner and Stadler (*27*) method as used by Rather (*40*). The colour of the extract solutions was found to be too deep to allow for any accurate determination of end point, even if phytin were present, but practically no noticeable precipitate was obtained from the iron added.

Attention was finally turned to a study of the dissolving of the inorganic phosphorus from the gummy magnesium mixture precipitate in the Forbes method (*18*). First acid alcohol was tried, then dilute nitric acid, and finally an 8 per cent nitric acid was used from the method of Bodnar (*8*) as the means of giving the greatest uniformity of results.

A tentative method. The method finally adopted was as follows:

Extraction of a five gram sample of hay in 250 cc. of 1.0 per cent hydrochloric acid solution for three hours, the bottle container being placed in a shaker to keep the sample thoroughly distributed throughout the liquid.

The sample was then filtered through a gooch crucible and 50 cc. of the filtrate taken for analysis. 40 cc. of magnesium mixture was added and 20 cc. of concentrated ammonium hydroxide, and the solution was allowed to stand for 48 hours.

The solution was next filtered through a buckner, taking the precautions mentioned above of allowing the filter pores to absorb some of the precipitate before suction was applied. Qualitative filter paper was used as the quantitative was found to be almost out of the question, the material refusing to filter.

The precipitate was then washed with 2.5 per cent ammonium hydroxide, and finally some 95 per cent alcohol. The filter paper and precipitate were removed to an Erlenmeyer flask, 25 cc. of 8 per cent nitric acid added, and with a stirring rod the paper and precipitate were broken up as much as possible; then the mixture was occasionally shaken for a period of 20 to 30 minutes, the solution filtered, washing the precipitate remaining on the filter carefully with water.

The volumetric analysis for phosphorus was run on this filtrate in the ordinary way. This modification of the above methods was adopted for a time as giving the most uniform results.

Work was continued, using this method on the 1926 crop of mixed hay samples. Three fractions were determined, (1) the inorganic phosphorus by the method above described, (2) the total extract which includes the inorganic phosphorus plus some organic phosphorus, by evaporating the solution to dryness in an evaporating dish after adding some calcium acetate to the solution, then burning, and running the regular volumetric phosphorus determination on the residue, and (3) the total phosphorus of the hay sample by ashing with magnesium nitrate and running phosphorus volumetrically. This method, though giving fairly accurate results, was found to be exceedingly slow where a large number of hay samples were to be analyzed.

Following the summer of 1927, in which a large number of additional samples were taken for analysis, it was felt that some quicker method was

necessary if the work was to be accomplished in any reasonable length of time, so an attempt was again made to apply some colorimetric methods to the problem. A colorimeter was procured that was similar in construction to the Bausch and Lomb instrument in which two extra cups were attached to allow for compensation of colour that might be present in the solution being analyzed. By carefully adding some of the dilute extract solution plus a small amount of the ammonium molybdate solution used in the Fiske and Subbarow method (*17*) to the compensating cup, a reading of the solutions could be accurately obtained. This method was then introduced instead of the Forbes method previously used and the entire group of samples of both 1926 and 1927 analyzed by it.

The final method. The Fiske and Subbarow method depends on the fact that certain reducing agents will produce a blue compound with the ammonium phospho-molybdate compound, and this colour in the unknown is then compared with a standard solution.

The inorganic, total extract and total phosphorus fractions were run as before, but using the colorimetric instead of volumetric method.

Reagents for the Fiske and Subbarow Method. Molybdate solution No. 1: 2.5 per cent ammonium molybdate in 5 normal sulphuric acid. Dissolve 25 grams of the salt in 200 cc. of water. Rinse into a litre flask containing 500 cc. of 10 normal sulphuric acid. Dilute, make up to volume, and mix.

Molybdate solution No. II: 2.5 per cent of ammonium molybdate in 3 normal sulphuric acid. Dissolve 25 grams of the salt in 200 cc. of water. Rinse into a litre flask containing 300 cc. of 10 normal sulphuric acid. Dilute, make up to volume, and mix.

Amino napthol sulphonic acid 0.25 per cent: Dissolve 0.5 grams of the dry powder in 195 cc. of 15 per cent sodium bisulphite, add 5 cc. of 20 per cent sodium sulphite, stopper and shake until dissolved.

Standard phosphate solution: 5 cc. = 0.4 Mgs. P. Weigh out 0.3509 grams of pure monopotassium phosphate in water, add 10 cc. of 10 normal sulphuric acid and dilute to a litre.

The procedure in the method finally adopted was as follows: Shake 5 grams of hay in 250 cc. of 1.0 per cent hydrochloric acid for 3 hours. Take a portion of this extract, depending on the quantity of phosphorus in the material, place in a 100 cc. volumetric flask, dilute to 75 cc. add 10 cc. of molybdate II solution, mix, then add 4 cc. of the amino napthol sulphonic acid, shake and allow to stand 10 minutes, then dilute to the mark, mix and compare with the standard.

The molybdate II solution is used for the inorganic determination as it is lower in acid and hence is less liable to hydrolyse organic compounds, and the molybdate I is used for the standard and all samples where the organic matter has been removed.

Test of method for extraction and hydrolysis. Forbes (*18*), in his work on the method for inorganic phosphorus, decided that three hours was sufficient time for extraction of the inorganic and phytin fractions of phosphorus. Anderson (*4*) used the same period for his work on inorganic

phosphorus. In order to be certain that the time required for the extraction of inorganic phosphorus and phytin, used by Forbes and Anderson, was sufficient, when analyzing hay samples, it was deemed advisable to check the procedure. Alfalfa hay was used as being fairly typical of the hays and the time required for complete extraction, studied together with the effect of the acid solution on hydrolysis of the organic phosphorus fraction over different periods of time.

TABLE 1. *Inorganic P_2O_5 determined on five gram samples of alfalfa hay, extracted for different periods of time in 250 cc. of 1 per cent hydrochloric acid (per cent.)*

Samples	1 Hour	2 Hours	3 Hours	5 Hours	12 Hours	24 Hours	48 Hours
1	.52	.51	.52	.52	.52	.52	.58
2		.52	.52	.52	.52		
3			.51	.51	.52		
4				.51	.52	.52	.58
5					.52	.53	.57

Total Phosphorus in Extract = .639.

Phosphorus was determined on five sets of duplicate samples extracted for different periods of time, as shown in the above table The five sets of samples were placed on the shaker at the same time. Set No. 1 was removed at the end of 1 hour, set No. 2 at the end of the second hour and sets 3, 4 and 5 at the end of the 3 hour shaking period. Sets No. 1, 2 and 3 were analyzed for phosphorus as soon as removed from the shaker, and then set aside at room temperature and analyzed at the intervals shown in the above table. Sets No. 4 and 5, however, were not analyzed immediately on removal from the shaker, but were set aside at room temperature and analyzed at the intervals shown.

These data indicate that total extraction may be expected after 1 hour's shaking, the three hours used being sufficient, and that no hydrolysis of the organic portion occurs within 12 to 24 hours at least.

Since all subsequent determinations were carried out within an hour of their removal from the shaker, it seems safe to assume that there is no danger of hydrolysis.

Test of the method for the recovery of added inorganic phosphorus. The extract solution when evaporated and the residue ashed, gives a higher percentage of phosphorus than can be determined directly on the extract. It seems safe to assume from the work of Forbes and Anderson that the extract solution may contain a portion of the organic phosphorus. Experiments were carried out to prove that the part determined directly on the extract solution is inorganic phosphorus. For this purpose inorganic phosphorus was added to the samples in varying amounts, and the resulting solutions analyzed. Monopotassium dihydrogen phosphate was added in a one per cent acid solution. 250 cc. of 1.0 per cent acid solution were used for extraction and the additions of phosphorus added to the samples prior to the shaking, care being taken that the extracting solutions were in all cases 1.0 per cent acid solutions, regardless of the phosphorus added.

TABLE 2. *Recovery of added phosphate.*

	P_2O_5 Added Mgs.	Mgs. of P_2O_5 inorganic present in 1 gram of hay computed	Mgs. of P_2O_5 inorganic recovered from 1 gram of hay determined	Per cent of total P_2O_5 present recovered
Alfalfa	Check	6.63	6.63	100.
"	1.13	7.76	7.93	102.0
"	2.21	8.48	8.59	101.0
"	2.57	9.20	9.15	99.5
Timothy	Check	1.34	1.34	100.0
"	1.43	2.77	2.74	99.0
"	1.80	3.14	3.18	101.0

This experiment shows that the phosphoric acid added to a hay sample, going through the shaking or extraction, can be recovered in the inorganic fraction by the colorimetric method.

Plimmer (*33*) suggested that the phytin in the seed was present as the calcium magnesium salt, but that it was transported as the potassium salt. No one has attempted to suggest the form in which the inorganic fraction is held in the hay, but as even aluminum phosphate is soluble in 1.0 per cent hydrochloric acid, there is little question of the solubility of any inorganic form that may be present.

A comparison of the tentative volumetric method and the final colorimetric method. As stated above, the mixed timothy and alsike samples were run by the Forbes method and also by the colorimetric method and it is interesting to note the comparison of the two methods on the following samples:

Sample No. 1 is from light yielding check plots, No. 2 from heavy yielding check plots, Nos. 3, 8 and 7 from plots receiving 100, 500 and 2,000 pounds per acre respectively of phosphate fertilizer.

TABLE 3. *Comparison of the volumetric and colorimetric methods of analysis for phosphorus. (Per cent).*

Sample No.	Volumetric Method			Colorimetric Method		
	Total P_2O_5	Total Extract P_2O_5	Inorganic P_2O_5	Total P_2O_5	Total Extract P_2O_5	Inorganic P_2O_5
1	.70	.40	.26	.67	.45	.28
2	.61	.39	.27	.61	.33	.27
3	.72	.53	.41	.79	.52	.44
8	.92	.70	.54	.91	.71	.54
7	1.13	.82	.64	1.13	.80	.61

The two methods, though not showing identical results, check close enough to give in this type of work comparative results. There is no comparison, however, in the time and labour of the two methods, the colorimetric being by far the quicker method. It is also my belief that the colorimetric method yields far more accurate results. The range of variation between duplicate determinations is much closer when the colorimetric method is used.

Parker and Fudge (*32*), in their work on phosphorus, state that the Fiske and Subbarow method proved satisfactory in the determination of phosphorus on the acid extracts of soil; however, no mention is made of this method being used for total soil phosphorus.

In the above work on the phosphorus of hay, the colorimetric method, compared with the volumetric method, proved so much more rapid and accurate, that an attempt was made to modify the method in such a way that it might be used in the routine total soil phosphorus determinations.

A group of soil samples was analyzed by the two methods and it was found that the colorimetric method proved much superior in time required for the determinations; at the same time the duplicates gave better checks than obtained by the volumetric method, and the percentage phosphorus determined by the two methods gave comparative results. The colorimetric method proved so much more rapid with equal accuracy that we are now using it for total soil phosphorus in the soils laboratory.*

The routine procedure is as follows: Weigh out 4 grams of soil in a porcelain crucible, mix thoroughly with 4 grams of magnesium nitrate. and heat slowly until the water of crystallization has been removed. The addition of a small piece of parawax will prevent bubbling while driving off the water. When dry, heat to 600°C. in an electric oven. Remove the contents of the crucible to a beaker and pulverize, rinse the crucible with 10 cc. of concentrated hydrochloric acid, and add to the sample in the beaker. Place the concentrated acid mixture on a steam bath, being careful that all the sample is moistened with acid, and digest for two hours. After the two hours on the bath add 25 cc. of distilled water and digest for 1.5 hours, again being careful that the soil mass is not adhering to the bottom of the beaker. Remove from the bath at the end of the second period and transfer the contents to a 200 cc. volumetric flask and make up to volume.

It is essential that care be taken to get all the iron into solution. If such care is not taken some of the iron may remain in colloidal solution and spoil the determination. As small a quantity of concentrated hydrochloric acid as will wet the sample is used, since too great a concentration of acid hinders the colour development later.

A sample (10 cc. to 50 cc. depending on the percentage phosphorus in the soil), usually 25 cc., is next transferred from the 200 cc. aliquot to a 100 cc. volumetric flask, add 10 cc. of molybdate I solution, and 5 cc. of the reducing solution, allow the colour to develop, make up to volume and compare in a colorimeter with the standard as described in the method on hay phosphorus.

Colour development may be retarded by the concentration of acid in some cases. This may be speeded up by raising the temperature 10°C. above the ordinary room temperature.

Problem.

The problem, as stated in the introduction, was the study of the variations in the inorganic and organic fractions of phosphorus in hays grown under different phosphate fertilizer treatments. A rechecking of methods employed in the analysis of the above fractions, established the fact that no satisfactory method existed for this type of work, and the work described under methods was performed, as a result of which a colorimetric

*University of Alberta.

method has been developed. The following experimental results were obtained by this method.

The work was originally started with the idea of studying the alsike-timothy crop on the peat land of Coon Creek Experiment Station, which showed decided increase in the percentage of phosphorus in hays under phosphatic fertilizer treatment as well as an increased yield of hay over the check plots.

Eckles, Becker and Palmer (*13*), in a bulletin on mineral deficiency of cattle, report a condition in cattle in Minnesota, also common in many parts of the world, that is believed to be due to some deficiency in the mineral portion of the ration; at least the condition found in Minnesota responds to the feeding of inorganic phosphorus, or high phosphorus hays. This condition in the State, along with the practice of growing hays on the peat lands, much of which is found by Alway (*1*) of the Soils Department to be low in phosphorus, led to the desire to see in what form these hays stored the phosphorus supplied in the form of fertilizer.

No experiments have been carried out that prove the ability of an animal to survive on an entirely inorganic phosphorus ration; however, it has been proven fairly conclusively that phosphorus supplied in the inorganic form to animals will suffice to overcome any deficiency of this element that may occur in the average diet. Without going into the discussion of whether organic or inorganic phosphorus is the better form of phosphorus for feeding, it would seem that if it could be proven that the increase in phosphorus in a pound of hay was entirely inorganic, it would be possible to advise the farmer to fertilize a field with an application of phosphorus heavy enough to produce his maximum paying crop of hay, and if this hay were found too low in phosphorus for a cattle ration it could be supplemented much easier and quicker by feeding the animal some form of inorganic phosphate directly. Phosphorus fed in this form would be the same to the cattle as phosphorus fed through a hay crop as inorganic phosphorus, and it would be a much cheaper method of getting it to the animals.

The phosphate hunger, according to Alway (*1*), is not by any means confined to the peat lands in Minnesota but is also found on the dark prairie soils of the West and Southwest, and the growing of hay on the latter soils is a problem somewhat similar to the one on peat in so far as they both respond to phosphate fertilization.

In dealing with hay samples such are used in this work, a study was made as nearly as possible of the forms of phosphorus as they exist in the average hay fed throughout the country. It is true that these may not be the forms in which it is to be found in a growing plant, but except in the case of pasture, the animal takes the phosphorus as it is provided in a hay, hay that is cured under various treatments and cut at various stages of its growth, and it is with such feeds that the farmer must deal in determining the ration for his stock.

The material. The data submitted consist of analysis of alsike hay and timothy hay grown on Coon Creek Experiment Station peat plots, rape from pot experiments on peat, alfalfa hay grown on the sandy upland

plots of Coon Creek, sweet clover, barley straw and wheat straw from Alberta wood soil pot experiments, and a set of upland native hay samples collected from both Alberta and Minnesota native hays. The alsike and timothy hay in 1926 was cut on Oct. 9th and analyzed as a mixed hay. The 1927 samples from the same plots, cut July 23rd, were separated and analyzed as individual samples. The alfalfa, cut May, 1927, was sampled about the time of the first cutting in Spring. Three set of samples of rape grown on peat, were used, two were early cuttings, collected when the plants had reached a height of 6 to 8 inches, the third were plants allowed to mature. The sweet clover, barley straw and wheat straw were supplied by the University of Alberta from experiments carried out on the fertilization of the podsolic wood soil.

Experimental data. The first three columns of the following tables give the amount of P_2O_5 in per cent reported to nearest second decimal in the three fractions, total sample, 1.0 per cent acid extract, and inorganic fraction. These percentages are based on the oven dried sample (100° in vacuum). The next two columns contain the phosphorus of first, the total extract, partly organic and partly inorganic, expressed as per cent of the total P_2O_5 of the hay, and second, the inorganic fraction expressed in the same way.

The organic P_2O_5 is expressed in terms of per cent of phosphorus in the hay, or in the same terms as the first three columns. These figures, expressed as per cent of total phosphorus, would be just the reverse of the inorganic columns so they are reported in such a way as to show the amount present per gram of hay.

Soluble organic and insoluble organic phosphorus have also been computed but these fractions show quite a variation between themselves and although they are given in the tables no discussion of them was considered necessary.

The data on the phosphorus fractions of mixed timothy and alsike hays grown in 1926 at the Coon Creek Experiment Station of the Division of Soils, subject to different phosphorus fertilization treatments, are given in table 4. These data indicate that under the increased phosphorus applications the inorganic fraction increased from 42.0 per cent of the total phosphorus on the check plots to 63.5 per cent on the fertilized plots. In the higher applications of fertilizer there is shown a slight increase of organic phosphorus, but hardly more than the general variations in this fraction, and this increase is shown on the plot with a 750 pound application of phosphate, the higher applications giving a slight decrease.

This hay is one that had gone through the entire season's growth, being harvested in October. The total P_2O_5 shows an increase of over 65 per cent, this increase being the difference between the check plot and the plot receiving the 750 pound application per acre of fertilizer.

The light and heavy checks mentioned in this table are two sets of check plots, those with the very light crop as light checks, and the heavy checks those with a better yield for some reason unknown. The heavy checks giving the greater number of pounds of hay are consequently somewhat lower in phosphorus per pound.

TABLE 4. *Phosphorus fractions on the mixed timothy and alsike hay from the Coon Creek Experiment Station,* 1926.

Sample No.	Pounds of phosphate fertilizer applied	Phosphorus fractions P_2O_5 (per cent). Total*	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insoluble organic
1	Light checks	.67	.45	.28	67.5	42.1	.39	.17	.22
2	Heavy checks	.61	.33	.27	53.9	43.7	.34	.06	.28
3	100 lbs.	.79	.52	.44	65.6	56.0	.35	.08	.27
5	200 lbs.	.83	.59	.49	71.1	58.7	.34	.10	.24
8	500 lbs.	.91	.71	.54	77.5	59.5	.37	.16	.21
10	750 lbs.	1.07	.79	.63	73.8	58.8	.44	.16	.28
4	1000 lbs.	.95	.71	.61	74.4	63.6	.35	.10	.25
7	2000 lbs.	1.13	.80	.61	71.2	54.0	.42	.19	.24

*Rate for P_2O_5 reported only to nearest second decimal.

The timothy and alsike hay grown on the mixed alsike and timothy plots in 1927 was harvested in July and the two hays separated by hand. The increase in total P_2O_5 on fertilized plots over check plots in the timothy, see table 5, ranges from 85 to 100 per cent. The inorganic phosphorus increases from 42.0 to 64.0 expressed as per cent of the total phosphorus. The organic fraction shows an increase in the 1,500 pound per acre application of fertilizer, but no increase under the 200 pound fertilizer treatment. The amount of organic phosphorus in this hay as compared with the mixed hay is just about half as much per pound of dry matter.

TABLE 5. *Phosphorus fractions on the Coon Creek timothy samples,* 1927.

Sample No.	Pounds of phosphate fertilizer applied	Phosphorus fractions P_2O_5 (per cent). Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insoluble organic
11	None.	.28	.17	.12	59.2	41.8	.16	.05	.12
12	None.	.27	.16	.11	60.7	41.2	.16	.05	.10
15	None.	.24	.15	.08	61.9	33.6	.16	.07	.09
16	None.	.22	.15	.09	67.1	38.3	.14	.06	.07
17	None.	.27	.22	.14	82.0	52.6	.13	.08	.05
13	200 lbs.	.44	.33	.28	75.5	64.0	.16	.05	.11
14	1500 lbs.	.53	.37	.32	70.5	61.5	.20	.05	.16

The alsike fraction of the mixed hay gives an increase of 175 per cent in total P_2O_5 as shown in table 6, and the increase in the inorganic fraction ranges from 30 per cent of the total P_2O_5 in the check plot to 60 per cent under high fertilizer treatment. Here again, as in the timothy, there is an increase noticeable in the organic fraction under the 1,500 pound per acre application of fertilizer. The value of 0.43 for percentage of organic phosphorus in the alsike clover under the 1,500 pound treatment of fertilizer cut in July, 1927, is very close to the value for the same fraction throughout the mixed hay samples cut in the fall of 1926.

The alfalfa samples from the upland plots at the Coon Creek Experiment Station were collected May 31st, 1927. These samples show a 34 per cent increase in total phosphorus in the treated plots as compared with the check plot, see table 7, and an increase of 48 to 62 per cent of the total P_2O_5 in the

inorganic fraction, the organic fraction showing very little change throughout the series.

TABLE 6. *Phosphorus fractions on alsike clover from the Coon Creek peat plots,* 1927.

Sample No.	Pounds of phosphate fertilizer applied	Phosphorus fractions P_2O_5 (per cent). Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insoluble organic
11	None.	.40	.24	.13	61.4	33.6	.26	.11	.15
12	None.	.41	.21	.12	52.1	30.0	.29	.09	.19
15	None.	.46	.33	.20	71.0	43.5	.26	.13	.13
16	None.	.44	.24	.14	55.2	32.1	.30	.10	.20
17	None.	.50	.33	.20	66.0	41.1	.29	.12	.17
13	200 lbs.	.56	.37	.29	66.6	51.2	.27	.09	.19
14	1500 lbs.	1.11	.90	.67	81.5	60.8	.43	.23	.21

TABLE 7. *Phosphorus fraction of alfalfa from the Coon Creek upland plots,* 1927.

Sample No.	Pounds of phosphate fertilizer applied	Phosphorus fractions P_2O_5 (per cent). Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insolubl organic
18	None.	.83	.47	.40	56.5	48.1	.43	.07	.36
20	100 lbs.	.79	.49	.41	62.1	51.9	.38	.08	.30
19	200 lbs.	.88	.57	.45	64.3	51.4	.43	.11	.32
21	400 lbs.	.89	.65	.54	73.0	61.1	.35	.11	.24
22	600 lbs.	.98	.68	.56	68.9	56.5	.43	.12	.31
23	1000 lbs.	.99	.67	.55	67.5	55.1	.45	.12	.32
24	1500 lbs.	1.01	.74	.63	73.5	62.5	.38	.11	.27
25	2000 lbs.	1.11	.81	.68	73.4	61.0	.43	.14	.30

The data listed in table 8 from a series of rape samples grown for 8 weeks on Coon Creek peat as pot experiments, show an increase of 130 per cent in total P_2O_5, the inorganic fraction increasing from 45.0 per cent total P_2O_5 on the checks to 69.0 per cent on the heavy treated plots; here again the organic fraction shows a slight increase over the check plot, but slightly lower than the plot with the 100 pound per acre application of fertilizer, so that it can be said that the high application of fertilizer in this case does not increase the organic phosphorus fraction to any appreciable extent.

The data on the mature rape plants, table 9, indicate a tremendous increase in total P_2O_5, practically 300 per cent. The inorganic increases from 63.0 per cent on the check plot to 83.0 per cent on the fertilized plots, higher than any of the previous crops. In this series again the higher fertilizer applications seem to give a certain increase in the organic fraction, but the increase is slight compared to the P_2O_5 present, while the 500 pound per acre application of fertilizer gives a higher organic fraction than the 2,000 pound treatment.

Another series of rape samples, table 10, grown on Alberta peat soil pot experiment for a period of 8 weeks, gives very similar results to the series on Minnesota peat, table 8.

TABLE 8. *Phosphorus fractions on an early cutting of rape,* 1927.

		Phosphorus fractions P_2O_5 (per cent).							
Sample No.	Pounds of phosphate fertilizer applied	Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insoluble organic
26	Check	.51	.32	.23	62.8	44 9	.28	.09	.19
27	100 lbs.	.87	.65	.49	74.5	56.3	.38	.16	.22
28	200 lbs.	1.00	.83	.62	83.0	62.1	.38	.21	.17
29	500 lbs.	1.08	.86	.72	79.9	66.2	.27	.15	.22
30	1000 lbs.	1.15	.97	.82	84.5	71.2	.33	.15	.18
31	2000 lbs.	1.18	1.01	.81	85.5	68.4	.37	.20	.17

TABLE 9. *Phosphorus fractions on a series of matured Rape samples,* 1927.

		Phosphorus fractions P_2O_5 (per cent).							
Sample No.	Pounds of phosphate fertilizer applied	Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insoluble organic
32	none.	.50	.37	.32	73.0	62.9	.19	.05	.14
33	100 lbs.	.79	.66	.55	83.5	69.2	.24	.11	.13
34	200 lbs.	.99	.89	.81	89.1	81.4	.19	.08	.11
35	500 lbs.	1.52	1.34	1.13	88.4	74.4	.39	.21	.18
36	1000 lbs.	1.98	1.82	1.64	91.8	81.8	.34	.18	.16
37	2000 lbs.	1.97	1.75	1.63	88.8	82.7	.34	.12	.22

TABLE 10. *Phosphorus fractions on some early cuttings of Rape, Alberta,* 1928.

		Phosphorus fractions P_2O_5 (per cent).			
Sample No.	Pounds of phosphate fertilizer applied	Total	Total Inorganic	Inorganic as per cent of total	Total organic
53	None.	.64	.29	44.3	.36
54	100 lbs.	.80	.40	50.5	.40
55	250 lbs.	.98	.52	53.5	.46
56	750 lbs.	1.06	.57	53.2	.49
57	1000 lbs.	1.20	.76	63.0	.54
58	2000 lbs.	1.24	.96	77.0	.28

A number of native hays were collected from different parts of the country, four from Alberta, and one from Minnesota. These were analyzed with a view to determining the variation, if any, that may occur in hays under different soil and climatic conditions. The hays have a low content of total phosphorus as shown in table 11, about 50 per cent of which is organic.

The samples from a series of wheat, sweet clover and barley experiments carried out in Alberta on podsolic or bush land soils under lime and phosphorus applications were analyzed (see table 12). These experiments were carried on to study the effect of lime and phosphorus on the above crops, as these soils in general respond to this treatment. The samples were supplied by the Soils Department, University of Alberta, for this work. The sweet clover, barley straw and wheat straw, show decided increase in inorganic phosphorus. The straws give a higher inorganic phosphorus fraction than the legume. The organic fraction seems to increase in the case of the wheat

straw and clover but not in the barley. The increase of total P_2O_5 in all cases averages around 100 per cent for the treated plants. The grains from the same plots show very little variation of the treated over the untreated; in all fractions, however, the organic fraction is by far the larger portion of the phosphorus.

The results in table 13 show the phosphorus fractions on a number of Minnesota native upland hays, slough hays, and cultivated alfalfa and timothy. The hays were collected in connection with the study of mineral deficiency in feeds and the condition of cattle as noted in the districts from which the hay was obtained, is given in the table. These hays on the whole are low in phosphorus, even the alfalfa samples, but there does not seem to be any correlation between the individual fractions such as inorganic as shown in this table, and the report of the condition among the stock.

TABLE 11. *Phosphorus fractions on Alberta native hays.*

		Phosphorus fractions P_2O_5 (per cent).							
Sample No.	Description or treatment	Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insoluble organic
38	A Minnesota prairie hay.	.13	.10	.07	73.3	49.6	.07	.03	.03
39	Alberta bush soil upland hay.	.33	.26	.22	78.9	67.5	.10	.04	.07
40	Peat hay in bush country.	.15	.13	.05	82.8	35.1	.10	.07	.03
41	Dry plains prairie hay.	.18	.13	.08	69.9	44.2	.10	.05	.05
42	Plains hay (transition)	.20	.20	.10	98.0	50.3	.10	.09	.01

TABLE 12. *Phosphorus fractions on a series of sweet clover, wheat, and barley.*

		Phosphorus fractions P_2O_5 (per cent).							
Sample No.	Description or treatment	Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic	Soluble organic	Insoluble organic
43	Sweet clover (check)	.28	.16	.11	56.7	37.6	.18	.05	.12
44	Sweet clover (Ca + P)	.66	.50	.39	75.4	59.4	.27	.11	.16
45	Wheat straw (check)	.22	.20	.18	88.0	79.0	.05	.02	.03
46	Wheat straw (Ca + P)	.56	.51	.49	91.5	88.4	.06	.02	.05
47	Barley straw (check)	.44	.39	.31	89.6	71.5	.12	.08	.05
48	Barley straw (Ca + P)	.89	.83	.78	93.8	87.5	.11	.05	.06
49	Wheat grain (check)	1.18	1.02	.08	86.2	6.7	1.10	.94	.16
50	Wheat grain (Ca + P)	1.17	1.04	.14	89.0	12.1	1.03	.90	.13
51	Barley grain (check)	1.31	.66	.15	50.6	11.5	1.26	.51	.65
52	Barley grain (Ca + P)	1.30	.69	.07	52.8	5.16	1.23	.62	.62

TABLE 13. *Phosphorus fractions on some Minnesota native hays.*

Sample No.	Kind of hay	Condition of cattle in area	Phosphorus fractions P_2O_5 (per cent). Total	Total Extract	Total Inorganic	Extract as per cent of total	Inorganic as per cent of total	Total organic
1014a	Alfalfa (Timber Soil)	Good	.70	.50	.41	72.0	58.0	.29
1006a	Alfalfa (Prairie Soil)	Bad	.41	.38	.23	91.6	57.2	.18
1013a	Alfalfa (Timber Soil)	Bad	.52	.39	.32	75.4	60.5	.21
1003a	Alfalfa (Prairie Soil)	Bad	.59	.33	.13	54.9	22.2	.46
1007a	Alfalfa (Timber Soil)	Bad previously	.46	.32	.27	68.5	57.5	.20
1007b	Alfalfa (Timber Soil)	Bad previously	.46	.32	.26	69.4	56 0	.20
1034a	Alfalfa (Prairie Soil)	Bad	.41	.23	.20	56.5	48.0	.21
1001a	Alfalfa (Prairie Soil)	Bad	.42	.23	.18	54.7	44.8	.23
1017	Alfalfa (Prairie Soil)	Bad	.28	.15	.09	51.0	32.1	.19
1020	Alfalfa (Timber Soil)	Slight trouble	.48		.29		60.5	.19
1013b	Alfalfa	Good	.46		.22		48.2	.24
1029a	Alfalfa, (Clay soil)	Fairly good	.35	.21	.13	59.3	37.8	.22
1029b	Wild hay	Bad	.40	.31	.22	76.9	54.5	.18
1001b	Wild hay, Prairie	Bad	.31	.23	.20	74.0	62.8	.12
1008	Wild hay, Prairie	Bad	.22	.16	.11	70.3	49.5	.11
1022b	Wild hay, Timber	Good	.24	.15	.09	63.8	39.1	.15
1018b	Wild hay, Prairie	Bad	.17	.13	.07	76.5	39.8	.11
1002	Wild hay, Prairie	Good	.26		.13		48.5	.13
1010	Wild hay, Prairie	Bad	.22	.14	.09	64.5	43.5	.13
1018c	Wild hay, Prairie Slough	Bad	.26	.17	.14	63.2	52.3	.13
1009	Wild hay, Prairie	Bad	.14		.07		51.8	.07
1033a	Wild hay, Prairie	Bad	.19	.11	.06	57.8	33.2	.13
1026	Wild hay, Prairie	Slight trouble	.13	.06	.04	48.0	27.1	.09
1028	Wild hay, Lake Bed	Slight trouble	.18		.13		71.2	.05
1025	Wild hay, Near Marsh	Bad	.18	.09	.05	47.8	23.4	.14
1032a	Wild hay, Prairie	Bad	.15	.04	.03	27.5	20.9	.12
1027	Wild hay, Lake Bed	Good	.16	.08	.04	51.5	27.0	.12
1016	Wild hay, Timber	Good	.15	.09	.06	55.2	35.7	.10

In the unfertilized hays the percentage of organic is generally higher than in the fertilized plots because of the increase in the inorganic fraction under fertilization. Legumes seem to be higher in organic P_2O_5 than prairie hays but they are also higher in total P_2O_5.

DISCUSSION

Graphical representation of data

The relationship of the three fractions, total, inorganic and organic phosphorus, is shown in figures 1, 2, and 3.

In all crops except the alsike clover and fall or late cutting of rape, figure 1, the total phosphorus of the hays shows a very rapid increase up to about a 200 pound application per acre of phosphorus fertilizer; from this point there is a continued gradual increase. The rape late cutting shows a similar curve but the increase continues to rise rapidly until 1000 pounds of fertilizer have been applied, and at this point the maximum seems to have been obtained. The alsike hay might show a somewhat different curve if a few more values had been plotted between the 200 pound application of phosphate and the 1500.

The curves for inorganic phosphorus, figure 2, are similar to those in figure 1, resembling the increase for total phosphorus very closely except the Alberta rape. This curve seems to show a general increase in inorganic throughout. Alsike has the same type of increase here as shown in total phosphorus.

In figure 3 quite a different set of curves is found for the organic fraction of phosphorus. Timothy, alsike, alfalfa, and the mixed hay samples show a decrease in total organic with the lower applications of phosphorus fertilizer as compared to the check plots, and in the higher treatments a slight increase. The three rape samples show an increase from the check plot to about the 1000 pound application and then a flattening out of the curve. The late cutting rape shows a point under the 2000 pound application that was so low in organic as to appear more or less out of line with the general trend of this fraction, so much so that the curve for this crop has not been extended to this point but was drawn only as far as the 1000 pound fertilizer application, to which point the curve follows the same trend as the other rape crops.

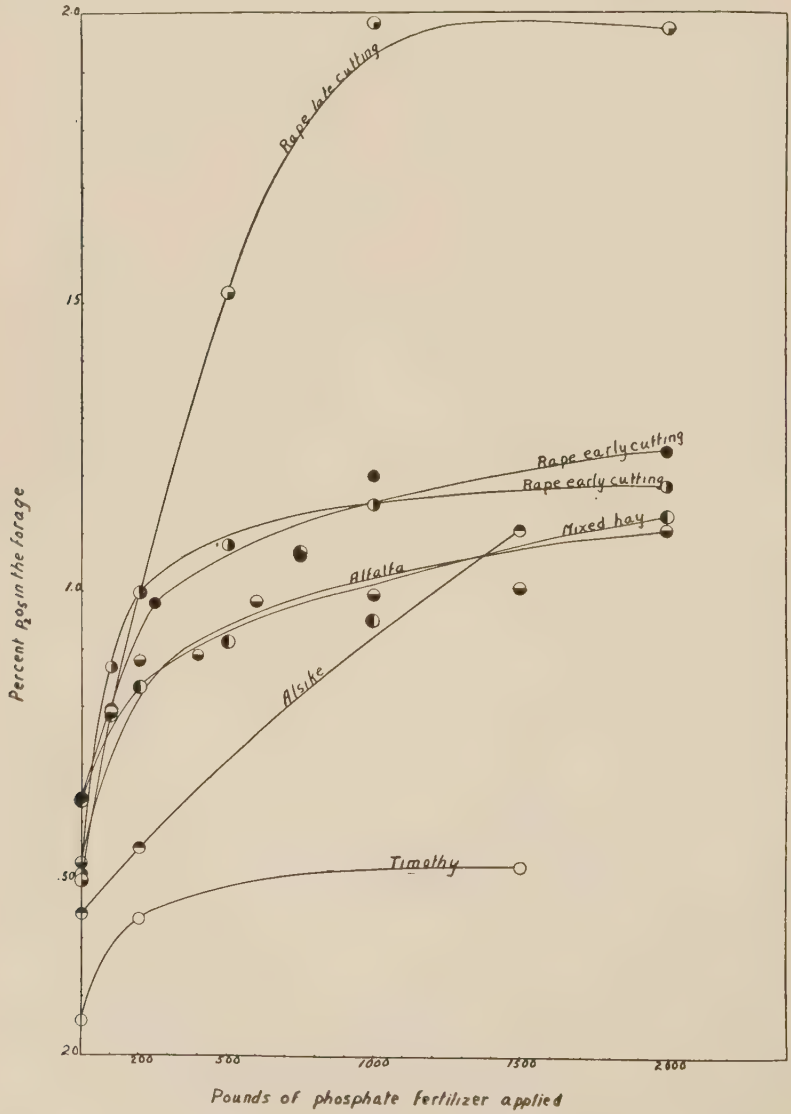

Figure 1. Showing the increase of total phosphorus in hays under increasing amounts of phosphate fertilizer in the soils.

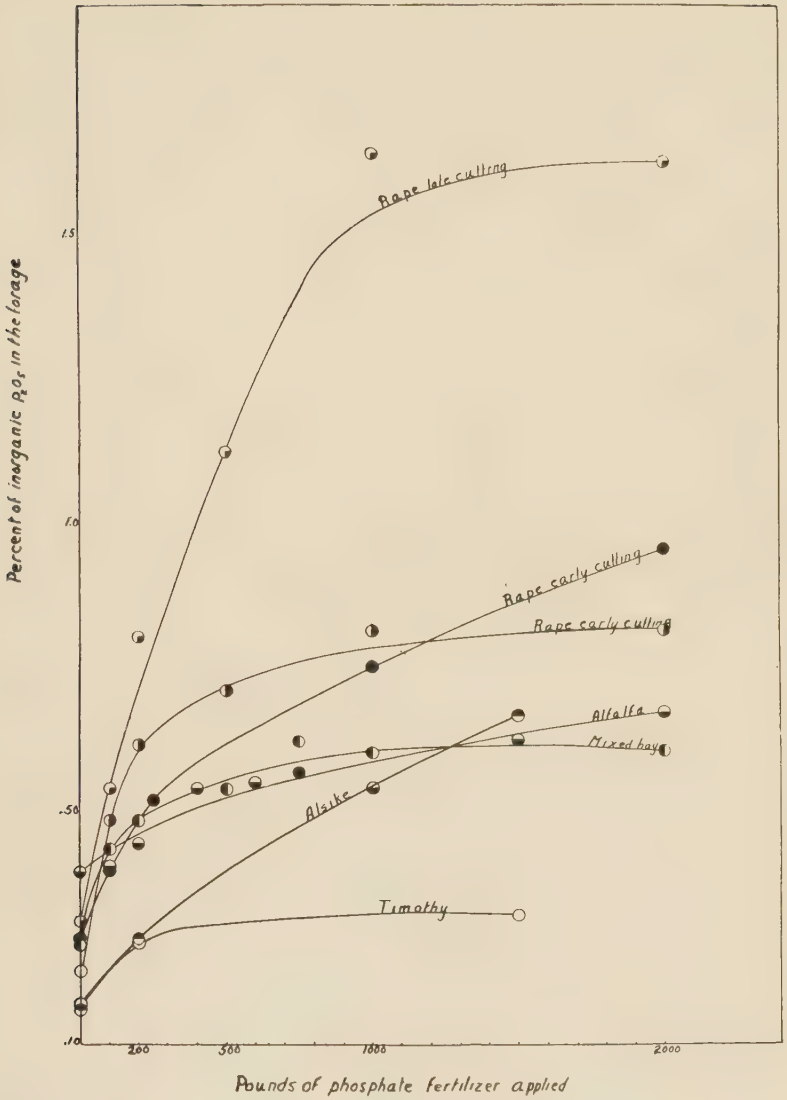

Figure 2. Showing the increase of inorganic phosphorus in hays under increasing amounts of phosphate fertilizer to the soil.

The values do not by any means fall on a smooth curve, but it is believed that the curves show quite clearly, when drawn as close as may be to the values given, the general trend of this fraction of phosphorus. The immediate increase of the organic phosphorus under the lower applications of phosphorus fertilizer seems peculiarly a characteristic of the rape samples only, while the other hays show quite a decrease at this point.

The graphs show fairly conclusively that the greater portion of the increase in phosphorus content of a hay is in the inorganic fraction. The only appreciable increase in organic phosphorus in actual pounds per acre of hay would be in the increase in pounds of hay per acre, and a maximum

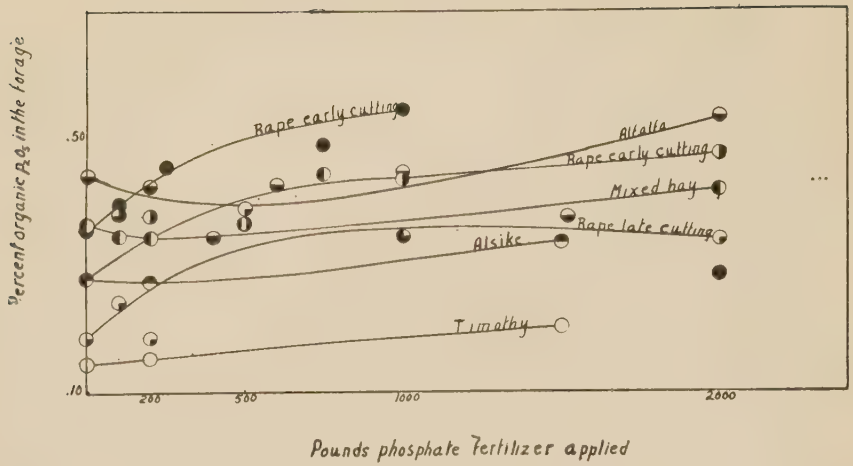

Figure 3. Showing the increase of organic phosphorus in hays under increasing amounts of phosphate fertilizer to the soil.

production of hay can readily be obtained under the 200 to 400 pound application of "Treble Superphosphate" fertilizer.

Statistical treatment of data.

In an attempt to bring out still further the points shown in the curves, the various fractions of phosphorus were correlated with the fertilizer applied, using the method of Harris (*22*), 1910.

The three rape series were combined and, in the case of the total phosphorus, correlated against fertilization $r = + .974 \pm .007$, bringing out the close relationship between the two. Where inorganic is compared with fertilization $r = + .591 \pm .061$, not quite as good a correlation as in the case of total, but showing a decided increase, nevertheless, with increased phosphorus to the soil. Where organic is correlated against fertilization $r = + .282 \pm .036$, bringing out as in figure 3 the slight increase in organic. The two fractions, organic and inorganic phosphorus were compared. The values $r = + .410 \pm .103$, indicate a certain increase in organic with increase in inorganic.

The peat hays, timothy and alsike of 1927 and the mixed hay of 1926 were compared in the same manner. Where total phosphorus of the hay is correlated with fertilizer application $r = + .483 \pm .163$, inorganic against fertilizer $r = +.591 \pm .061$, and organic against fertilizer $r = +0.34 \pm .240$. These results show a much lower correlation between total phosphorus and inorganic against fertilization, with a better correlation between organic and fertilization than in the rape series, though the probable error in this latter case is large enough to make the correlation of little significance. Correlating organic against inorganic $r = + .916 \pm .030$, a high correlation, showing a greater increase of organic per increase in inorganic than in any of the other series. The high correlation here is probably accentuated by the fact that in case of the timothy and alsike only two values apiece are available.

The alfalfa samples treated in the same manner, for total phosphorus against fertilizer $r = + .932 \pm .038$, inorganic against fertilization

$r = + .477 \pm .197$, organic against fertilizer $r = + .001 \pm .255$, and inorganic compared with organic $r = + .00003 \pm .255$. The total phosphorus shows a high correction value with fertilizer applied, the inorganic fraction due to the high probable error is of little value, but the organic fraction correlated with fertilizer and the two fractions, organic and inorganic, compared, show no correlation whatever.

General discussion of data. In general the Coon Creek crops show an increase in inorganic P_2O_5 of from 41 to 63 per cent, under increased fertilizer treatments, the organic fraction therefore decreasing from 59 to 37 per cent of the total P_2O_5. The actual amount of P_2O_5 in organic combination in these crops does not vary much, showing a slight increase in the timothy and alsike under the heavier treatments. The timothy hay is lower in total phosphorus, as are also the prairie wild hays, but the percentage of inorganic P_2O_5 of the total in the timothy crop is about the same as in the legumes.

It may be of interest in discussing these results to quote some of the earlier works on forages and compare the results with the above data. Much of the previous work done will not be discussed because of the difference in the earlier methods and the doubts expressed by Rather (*40*) and Forbes (*20*) as to the accuracy of the results from the use of these methods.

MacGillivray (*30*), in some work on tomato plants, states that there is a "re-utilization"* of phosphorus in plants, under phosphorus deficiency and the growth under such conditions is in the upper leaves, in this work confirming the work of André (*6*), who states there is greater redistribution of phosphorus in the plant than of other minerals. MacGillivray's data indicate an increase of phosphorus in the fruit towards ripening; this in turn has been found to be the case in seeds of plants.

It would appear, therefore, that the big increase in yield under the first applications of phosphorus might be due to a supply of phosphorus sufficient to carry on growth on a large leaf surface, instead of in local areas as mentioned by MacGillivray under phosphorus starvation. This being the case, the curing of a hay to prevent loss of a portion of the leaves would be quite important in harvesting.

Among forage plants, according to Forbes, leguminous hays and bluegrass are high in phosphorus, while timothy hay and wheat straw are low in this constituent. This is shown to be true when comparing the timothy of table 4 and the straws of table 12, with the legumes. He states that the content of phosphorus of all sorts of roughage is much affected by the soil. It would appear from the results of table 12 that this is much more true in case of the roughage than in the case of the grains.

Ames and Boltz (*2*) find first cuttings of alfalfa higher in total phosphorus in all cases than the second cuttings. They also state that the mineral elements are loosely combined in the forage plants and are, therefore, easily leached out by rain. They state that 0.5 inches of rain on an alfalfa crop may decrease its value 25 per cent due to leaching of inorganic constituents, and in an experiment they determine 75 per cent of the phosphorus of alfalfa

*Term used by MacGillivray to describe the translocation of phosphorus from the older tissues to the growing parts.

as soluble in distilled water, and they list 43 - 63 per cent of alfalfa phosphorus as organic, somewhat higher values than the 38 to 52 per cent found on the Coon Creek plots. They report that for every increased condition of fertilization, a greater proportion of the total phosphoric acid is present as organic phosphorus. This is not brought out in the alfalfa plots from Coon Creek. They believe alfalfa cut in the bloom to contain the largest amounts of the more valuable plant foods and nutritive constituents of the leaves.

Quite a variation in mineral constituents, including phosphorus throughout the growing season, is reported by different authors. Elliot, Orr, Wood and Cruikshank (*15*) report Leicestershire pastures as having their maximum nitrogen and phosphorus early in May, other fodders having their maximum in June. The Veld hays of South Africa as reported by Theiler (*48*), decrease steadily in phosphorus content from 0.6 per cent of P_2O_5 in November to .09 per cent in June. Crowther and Ruston (*12*) report the tendency of potassium and phosphorus to decrease as the season advances, while Fagen and Jones (*16*) found throughout the season that phosphorus rose to a maximum and then decreased. Woodman, Blunt and Stewart (*50*) report calcium in pasture rising to a maximum and then decreasing, but find phosphorus showing opposite behaviour.

From these references it may be gathered that there is a definite seasonal variation in the phosphorus content of fodders but there does not seem to be in all cases a definite trend in any one direction, though the concensus of opinion seems to favour a decrease as the season advances. In the the above tables the organic fraction of the fall cutting of mixed hay is higher than the hay cut the next year in July and the alfalfa cut on May 31, 1927 is as high in this fraction as the alsike timothy mixture cut in the fall and higher than the alsike cut in July. However, this is not a fair comparison, as the crops are different, though both legumes and the alfalfa is grown on upland while the alsike is grown on peat.

In the rape experiments the spring cutting, table 8, and the Alberta samples, table 10, may be said to show no increase of organic phosphorus under increased application of fertilizer. The fall cutting shows some increase under the higher applications but the 500 pound application gives the highest organic content so these differences may be small.

Silica content of hays. During the ashing of the hay samples for total phosphorus a difference was noticed in the insoluble ash of the timothy and legumes. A certain difference in the insoluble ash of the legumes under high and low phosphorus treatments was also observed, and some samples were analysed to determine the extent of this difference.

The hays were ashed and then treated with concentrated hydrochloric acid and the residue filtered, dried, burned and weighed.

The data on native hays show the large increase in silica on these kinds of hays as compared with the legumes or timonthy. Elliot, Orr, Wood and Godden (*14*), in their work on cultivated and uncultivated pastures, re-

TABLE 14. *Comparing the acid insoluble ash and the P_2O_5 of hays.*

Sample No.			Total P_2O_5 per cent	Acid Insol. Ash per cent.
T	17	Timothy check.	.27	3.22
T	14	Timothy, 1500 lbs. Fertilizer.	.53	2.02
A	17	Alsike check.	.50	0.40
A	14	Alsike, 1500 lbs. Fertilizer	1.11	0.31
	18	Alfalfa check	.83	1.05
	25	Alfalfa, 2000 lbs. Fertilizer	1.11	.50
		NATIVE HAYS		
	38	Prairie hay	.13	5.66
	39	Wild hay, timber	.33	8.24
	42	Prairie hay	.20	8.03
	41	Prairie hay	.18	7.46
	49	Peat hay	.15	5.21

port the cultivated pastures as giving the hay with the highest silica free ash, the wild hays being highest in silica.

Schollenberger (*44*), in some work on silica, shows that silica is assimilated by plants when added in available form, but he states that added silica did not help the plant utilize more phosphorus from a furnace slag, while silica from calcium silicate seemed to help the utilization of phosphorus. Most of his work was carried out as pot experiments, but on a field test he states that where silica was added as a slag, the phosphorus showed a decrease, though the yield increased. He believed this to be due to the high nitrogen content of the field rather than to any effect of the added silica.

These data on the timothy, alsike and alfalfa may indicate a certain utilization of silica under phosphorus deficiency. The timothy and alsike hays are from a peat soil while the alfalfa is from a sand. As much as 100 per cent increase may be seen in the case of the alfalfa.

Crude protein nitrogen of hays. Some further work was carried out on the alfalfa, alsike and timothy samples to study the effect of the fertilizer treatment on nitrogen metabolism. A number of samples were selected representing low and high phosphorus treatment of these hays.

MacGillivray's (*30*) work with tomato plants seems to indicate some relationship between the phosphorus and nitrogen of these plants. He states that plants in nutrient solutions under starved conditions respond much quicker to nitrogen fertilization than to phosphorus. Apparently a plant deficient in nitrogen can take up nitrogen from the solution at different stages of its growth and utilize it immediately, while it cannot utilize phosphorus quite so readily, if under phosphorus deficiency treatment and phosphate fertilizer is applied.

He concludes that nitrogen and phosphorus deficiency show certain points in common, such as stiffening of the stems, decrease of chlorophyll, appearance of anthocyanin pigments on the under side of the leaves; his results show embryonic regions, which are high in protein content, such as fruit clusters, stems, the tops of the leaves, and the tops of stems, to be high in both phosphorus and nitrogen.

Plants grown in sand in the absence of phosphorus show a greater percentage of nitrogen than plants having an ample supply of phosphorus. His plants grown in the absence of phosphorus show a decrease in the percentage total nitrogen and also the total amount of coagulable nitrogen, as compared with the coagulable nitrogen of plants grown under sufficient phosphorus treatment. Ames and Boltz (*2*) state in connection with their alfalfa experiment that 80 per cent of the protein of the plant is present in the leaves, the percentage of nitrogen, as well as phosphorus, is found highest in the first cutting of alfalfa, and more total nitrogen is combined as protein in the second cutting than in the first, 66 per cent in the first cutting and 77 per cent in the second being found as protein nitrogen. It would therefore appear that the nitrogen varies in much the same order as the phosphorus.

TABLE 15. *Table showing comparison of crude protein and phosphorus content of hays.*

Sample No.	Treatment	Per cent P_2O_5	Per cent crude Protein
Timothy 11	No treatment	.28	8.92
Timothy 12	No treatment	.27	8.83
Timothy 17	No treatment	.27	8.45
Timothy 14	1500 lbs. phos.	.53	7.88
Alsike 11	No treatment	.40	15.75
Alsike 12	No treatment	.41	15.22
Alsike 17	No treatment	.50	15.69
Alsike 13	200 lbs. phos.	.56	16.04
Alsike 14	1500 lbs. phos.	1.11	17.86
Alfalfa 18	No treatment	.83	28.20
Alfalfa 25	2000 lbs. phos.	1.11	28.69
Mixed hay 1	No treatment	.67	25.33
Mixed hay 2	No treatment	.61	25.78
Mixed hay 7	2000 lbs. phos.	1.13	23.44

The literature cited on this phase of the problem seems to show a relationship in practically all cases between the nitrogen and the phosphorus.

The above data on nitrogen of the Coon Creek hays show a decrease of crude protein in the high phosphorus timothy samples as compared with the checks. In the alsike grown on the same plots as the timothy the same year the crude protein shows an increase of about 14 per cent. The mixed hay timothy and alsike cut in the fall of 1926 shows a higher crude protein content throughout than the early cutting of alsike and timothy 1927, but the fertilized plots show a decrease in nitrogen as compared with the checks. The alfalfa crop is very similar in nitrogen content on check plots and under 2000 pounds phosphate fertilization.

In general these data show no general trend of the crude protein nitrogen fraction, certainly no direct relationship to the fluctuations in the phosphorus throughout the three groups.

SUMMARY

Various methods for the determination of inorganic phosphorus in the presence of organic phosphorus in plant tissues were compared, with a view to obtaining a rapid and accurate method for this type of analysis. A modification of the Fiske and Subbarow colorimetric method for phosphorus was

developed, which proved much more rapid than any of the older volumetric methods, and proved more accurate, due to the prevention of hydrolysis of organic phosphorus compounds which may occur in the more time-consuming methods.

This modified method proved accurate and rapid in the determination of total phosphorus in plant tissues, and with a few alterations proved very satisfactory as a method for the determination of total soil phosphorus.

Many samples of forage crops were examined to determine the extent to which total phosphorus increased in the plant tissues under various applications of phosphate fertilizer, and to what extent this increase, if any, occurred in the inorganic or the organic fractions. Very large increases in total phosphorus in plants were obtained on the plots receiving heavy fertilizer applications as compared to the check plots receiving no fertilizer. The increases in phosphorus found in the various crops expressed as per cents, were respectively: a mixed hay (alsike clover and timothy) cut in October, 69; timothy from the same plots cut in July, 89; alsike, 175; alfalfa samples from upland sandy soils, 34; two sets of rape samples cut at the end of six weeks growth, 130 and 94; sweet clover, 135; wheat straw, 150; barley straw, 100. An average of the various increases in the above crops gave 100 per cent increases, or a doubling of the phosphorus content, due to the application of fertilizer.

A sample of rape allowed to mature, gave a 300 per cent increase in total phosphorus. This value was not included in the above list as being above the average increase.

Practically the entire increase in total phosphorus of hays was confined to the inorganic phosphorus fraction, fertilization increasing the organic fraction only in so far as it increased the pounds of hay per acre.

Very heavy fertilizer applications were necessary to obtain these large increases of inorganic phosphorus in the plant. The fertilizer experiments show, on the other hand, that a maximum yield in pounds of hay per acre can be obtained with a 200 pound application of treble superphosphate, instead of the 1000 to 2000 pound applications necessary to give the extremely high phosphorus content obtained in these crops. It would seem, therefore, that phosphate fertilizer could be profitably applied only in so far as it increased the pounds of hay per acre, and that phosphate required in a ration in excess of the quantity supplied in such a hay could be added directly to the feed.

Where it was deemed necessary to supplement a ration with organic phosphorus, this could be done by feeding cereal grains rather than by attempting to increase the organic phosphorus content of forage crops by means of large applications of phosphate fertilizer. These grains contain high percentages of phosphorus, for the most part in organic combination, and they do not show the extreme fluctuations in percentage phosphorus which seem characteristic of forage plants.

The content of silica in the ash of the various hays appears to decrease with the application of fertilizer. The variation, in the case of alfalfa,

between the check plots and those receiving fertilizer was 100 per cent. The legumes in general from the check plots, did not exceed 1.0 per cent of silica, with decidedly less on the fertilized plots. Timothy contained 2.0 to 3.0 per cent of silica under all treatments, showing the largest percentages, however, on the check plots, while the native wild hays contained as high as 5.0 to 8.0 per cent.

Alsike showed an increase in protein under the higher phosphorus application, timothy and mixed hay a decrease, while alfalfa showed very little difference between the high and low phosphorus plots. In general no direct relationship was shown in the fluctuations of nitrogen and phosphorus in the series of samples worked with.

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